

Att'y Dkt: 4: 2722002621

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diseases. While topical administration is generally preferable for stimulating hair growth, as generally only local effects are desired, systemic treatment may be preferable in some instances as well.

5        Assays for Compounds Useful in the Invention

Assays for assessing the ability of a compound to inhibit proteasoma activity and for inhibitors of NF- $\kappa$ B activity are well known in the art. Two typical, but nonlimiting assays are described below.

10       Assessment of Proteasomal Activity

Proteasomal inhibition activity is most conveniently measured by the assay described in Example 5 hereinbelow. The assay involves incubating the potential inhibitor with 20S thermophila proteasomes which, in purified form, are commercially available, with a fluorogenic peptide substrate. The presence of an inhibitor will reduce the amount of fluorescence generated by the action of the proteasome fraction on the fluorogenic peptide. This assay is described in further detail in Coux, O. *et al. N Rev Biochem* (1996) 65:801; Adams, J. *et al. Cancer Res* (1999) 59:2615; and Caiu, A. *et al. J Biol Chem* (1997) 272:13437. Further reports are set forth in Hilt, W. *et al. Trans Biochem Sci* (1996) 21:96; Peters, J. *Trends Biochem Sci* (1994) 19:377; Maupin-Furlow, J.A. *et al. J Biol Chem* (1995) 270:28617; and Jensen, T.J. *et al. Cell* (1995) 83:129. Fluorogenic substrates and purified proteasomes are available, for example, from CalBiochem, San Diego, CA.

25       NF- $\kappa$ B Activity Assays

Cells are treated with different concentrations of compounds, and nuclear extracts prepared. Briefly, cells are washed with phosphate-buffered saline, and resuspended in lysis buffer (0.6% Nonidet P-40, 150 mM NaCl, 10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.5 mM DTT and a cocktail of protease inhibitors (Complete (TM), Boehringer Mannheim). After incubation on ice for 15 min, nuclei are collected by centrifugation.